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Glycosylated hemoglobin (HbA_1) and hemoglobinopathies in pregnancy*

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1 Introduction

Glycosylated hemoglobin (HbA_1) has been correlated with prior blood glucose levels and has been in the pregnant and non-pregnant diabetics as a possible index of both long and short term glucose control [7]. Factors other than blood glucose concentration have been reported to alter the value of HbA_1 [2]. Hemoglobinopathy is one of these [1]. This paper reports the results of a prospective and retrospective evaluation of HbA_1 in patients with "normal" and "variant" hemoglobin.

2 Materials and Methods

This study was carried out at Sloane Hospital for Women of Columbia-Presbyterian Medical Center. The patients belonged to obstetric clinic population, which comprises of 49 percent hispanics, 44 percent blacks, and 7 percent others. Earlier, the pregnant patients at high risk for diabetes were screened using plasma sugars (fasting and 2 hours post 100 gms oral glucose-load) and HbA_1 as screening parameters. The mean HbA_1 in patients with normal plasma sugars was 6.17 ± 0.6. Seventeen patients with HbA_1 value of 5 or less (2 S.D. below mean) were identified as study-group-experimental group. Another group of 17 patients, randomly selected and matched according to age, parity, gestational age and hematocrit with the first group; and HbA_1 above 5 gms. percent were selected as a control group. HbA_1 was also measured in a third group of six patients with known hemoglobinopathy. The patients in first two groups were investigated for the presence of hemoglobinopathy by careful review of their medical records, sickle cell solubility test or hemoglobin electrophoresis.

Hemoglobin A_1 determinations were performed by cation exchange column chromatography, using micro-column fast-hemoglobin test system (ISOLAB, Akron, Ohio). Columns and reagents were equilibrated at 22 °C–24 °C before performing and during the test. Blood specimens were collected in heparinized tubes. Specimens were tested, when freshly drawn. When this was not possible, a hemolysate was prepared and stored

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Curriculum vitae

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at 4 °C. All specimens were analysed within 48 hours. The distribution of the data was evaluated by ordered Chi square analysis and relative risk assessment.

3 Results

Of seventeen control patients with HbA_1 greater than 5 percent, 15 had HbAA or negative sickle cell solubility test and two had sickle cell trait. Of seventeen patients with HbA_1 less than 5 percent, 14 had sickle cell trait and two had C trait. The remaining patient had a negative sickle cell solubility test. The distribution of these cases is shown in Tab. I.

The mean HbA_1 in patients with documented variant Hb was 4.29 ± 0.59 (S.D.) and in 16 patients with HbA, 5.94 ± 0.72 (S.D.). The possibility of this distribution occurring by chance is less than one in a thousand ($X^2 = 23.18$, $p < 0.001$).

The percentage of HbA_1 in the third group of patients with known variant Hb is presented in Tab. II.

The HbA_1 level was remarkably low in a patient with Hb Sc disease, markedly elevated in a patient with S-HPFH (S-high persistence of fetal hemoglobin), high in thalassemia and Lepore Hb, and borderline in alpha 2 sickle cell thalassemia.

4 Discussion

Hemoglobin A_1 is produced by nonezymatic glycosylation of hemoglobin A which occurs slowly throughout the life-span of red blood cells [3]. Glycosylation of the N-terminal amino acid of the beta chain of HbA changes its mobility in cation exchange chromatography allowing separation from the parent hemoglobin molecule [3]. Other types of hemoglobin, (such as hemoglobin S, F or C), also have differing mobilities on cation exchange column chromatography. Hemoglobin F happens to elute in the same fraction as HbA_1, whereas hemoglobin S and hemoglobin C elute in different fractions [1, 7]. It has been shown that glycosylation occurs in other parts of both the alpha and beta chains of hemoglobin A, and presumably of other hemoglobins [5].

Only glycosylation of the N-terminal valine of the beta chain significantly changes its chromatographic mobility [5]. Although the alpha, beta and beta S subunits of a patient with sickle cell trait may be glycosylated, only hemoglobin with a glycosylated beta subunit will be measured in the HbA_1 fraction. The presence of total glycosylated hemoglobin in such a patient may, thus, be underestimated. This phenomenon has been previously reported in non-pregnant patient [6]. The significant difference in HbA_1 as measured by column chromatography between subjects with "normal" hemoglobin and those with hemoglobin AS and hemoglobin AC represents a difference in the presence of glycosylated beta chains rather than glycosylation per se. Perhaps, for the same reason, the patient with hemoglobin SC disease had a very low percentage of HbA_1 (Tab. II, not included in Tab. I).

In patients with thalassemia minor, there is a compensatory increase in fetal hemoglobin to make up for the defect in beta chain synthesis. There may be no increase in glycosylation. However, since the chromatographic mobility of fetal hemoglobin and

Tab. I. HbA_1 in Patients with "Normal" and "Variant" Hb

Percent HbA_1	Number of Patients with Hb AS or AC	Number of Patients with Hb AA	Total
> 5	2	15	17
< 5	16	1	17
total No. of patients	18	16	34
$X^2 = 23.18$	$p < 0.001$		

Tab. II. HbA_1 In Patients with Variant Hb (not included in Tab. I)

Hemoglobinopathy	Percent HbA_1
Thalassemia Minor	8.2
Thalassemia Minor	9.1
Hemoglobin SC Disease	1.5
Alpha 2 Sickle Cell – Thalassemia	5.1
S-HPFH (S-high persistence of fetal hemoglobin)	34
A-Lepore Hb (11 %)	6.9

HbA_1 are similar, the percentage of HbA_1 in these patients may be overestimated [7]. This explains high values of HbA_1, in the patients with high persistence of fetal Hgb and thalassemia minor. (Tab. II). The patient with alpha-2-thalassemia AS demonstrated the combined effect of the above two factors which caused a return of the estimation of HbA_1 to nearly "normal" values.

FLÜCKIGER and WINTERHALTER have described a colorimetric assay for the determination of glycosylated hemoglobin. The estimation of glycosylated hemoglobin obtained with their method is appropriate to the glucose level and it is not altered by variant hemoglobins [4]. Our data suggests that a HbA_1 value of less than 5 percent, as measured by cation exchange chromatography should alert one to the possible presence of hemoglobinopathy. In patients with apparently normal carbohydrate tolerance and elevated levels of HbA_1, thalassemia (persistent hemoglobin F) should be suspected. It should be recognized that in diabetics with sickle cell trait, the estimation of HbA_1 may fall in the normal range when measured by cation exchange column chromatography in spite of abnormal carbohydrate tolerance. However, decreasing values of HbA_1 are representative of better control of diabetes even in these patients.

5 Conclusion

HbA_1 is a valuable indicator of the integrated plasma glucose level over the previous 4 to 6 weeks, and therefore, a useful adjunct in the management of diabetic pregnancy [3]. The estimation of HbA_1 by cation exchange column chromatography enables one to identify patients with variant hemoglobins. However, obtaining a single value interferes with the assessment of diabetes in the patients with hemoglobinopathy. These technical problems could be overcome if the colorimetric method was used in the estimation of glycosylated hemoglobins.

Summary

Glycosylated hemoglobin (HbA_1) is considered to be representative of prior blood-glucose levels and is being used in pregnant and nonpregnant diabetic patients as a possible index of both long and short-term glucose-control. Factors other than blood-glucose concentration have been reported to affect its value. Variant hemoglobin is one of them. HbA_1 and blood-glucose levels were measured in pregnant patients at high risk for diabetes for screening for abnormal carbohydrate metabolism. HbA_1 was measured by cation exchange column chromatography and glucose was measured by hexokinase reaction. The mean HbA_1 in patients with normal blood sugars was 6.17 ± 0.6 percent. A value of HbA_1 of less than 5 percent as measured by cation exchange column chromatography was highly predictive ($P < 0.001$) of hemoglobinopathies (S or C). The mean HbA_1 of randomly selected matched patients with "normal" Hb was 5.94 ± 0.72 percent. In patients with thalassemia, HbA_1 values as measured by cation exchange column chromatography were elevated despite normal carbohydrate tolerance. While interpreting the results of HbA_1 in the management of pregnant diabetics, the above fact should be kept in mind.

Keywords: Glycosylatin, hemoglobin, hemoglobinopathy

Zusammenfassung

Glykosyliertes Hämoglobin (HbA_1) und Hämoglobinopathien in der Schwangerschaft

Es wird angenommen, daß das glykosylierte Hämoglobin (HbA_1) eine repräsentative Aussage über den Blutzuckerspiegel zuläßt. Man benutzt dieses Maß sowohl bei schwangeren Diabetikerinnen wie auch beim üblichen Diabetes als Index für die länger- wie auch kurzfristige Blutzuckerkontrolle. Es wurde jedoch berichtet, daß neben dem Blutzuckerspiegel andere Faktoren, wie z.B. das Varianthämoglobin, den HbA_1-Wert beeinflussen können. Wir bestimmten die HbA_1 – und Blutzuckerspiegel bei schwangeren Frauen, die mit einem hohen Diabetesrisiko behaftet waren, um pathologische Veränderungen im Kohlenhydratstoffwechsel aufzudecken. Das HbA_1 wurde säulenchromatographisch über einen Kationenaustauscher bestimmt; die Glucose haben wir mittels der Hexokinase-Reaktion gemessen. Bei Patientinnen mit normalem Blutzuckerspiegel betrug der mittlere HbA_1-

Wert 6,17 ± 0,6 %. Ein säulenchromatographisch bestimmter HbA_1-Wert von unter 5 % sagte mit hoher Wahrscheinlichkeit ($p < 0.001$) eine Hämoglobinopathie (S oder C) voraus. Der mittlere HbA_1-Wert von zufällig ausgewählten Patienten mit einem „normalen" Hb betrug 5,94 ± 0,72 %. Bei Patienten mit Thalassämie waren die HbA_1-Werte trotz ungestörter Glucose-Toleranz erhöht. Bei der Interpretation der HbA_1-Werte beim Schwangerschaftsdiabetes sollten diese Faktoren berücksichtigt werden.

Schlüsselwörter: Glykosylierung, Hämoglobin, Hämoglobinopathien.

Résumé

Hémoglobine glycosylée (HbA_1) et hémoglobinopathies pendant la grossesse
L'hémoglobine glydoxylée (HbA_1) est considérée comme représentative des taux de glycémies antérieures et est utilisée chez les patientes diabétiques en cours et en dehors de la grossesse en tant qu'index du contrôle de la glycémie à court terme aussi bien qu'à long terme. On a rapporté que son taux pouvait être modifié par d'autres facteurs que la glycémie. Les hémoglobines anormales représentent un de ces facteurs. On a mesuré l'HbA_1 et la glycémie chez des patientes enceintes à haut risque diabétique en raison de la découverte d'une anomalie du métabolisme glucidique. La mesure d'HbA_1 a été effectuée par chromatographie sur colonne d'échange cationique et la glycémie par une réaction à l'hexokinase. La valeur moyenne d'HbA_1 était de 6,17 ± 0,6 pour cent chez les patientes à glycémie normale. Une valeur d'HbA_1 inférieure à 5 pour cent si elle est déterminée par chromatographie sur colonne d'échange cationique est hautement prédictive ($p < 0,001$) d'hémoglobinopathies (S ou C). La valeur moyenne d'HbA_1 chez des patientes avec une hémoglobine «normale» appariées par randomisation est de 5,94 ± 0,72 pour cent. Chez les patientes thalassémiques les valeurs d'HbA_1 déterminées par chromatographie sur colonne sont élevées malgré une tolérance glucidique normale. Lors de l'interprétation des résultats d'HbA_1 au cours de la surveillance des diabétiques enceintes, les faits ci-dessus devraint être garés en mémoire.

Mots-clés: Glycosylation, hémoglobine, hémoglobinopathie.

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